# Formation, Structure and Function of Cartilage

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• **Improved investigative techniques including electron microscopy, isotope tracings and improved histochemistry have greatly increased knowledge of the function of cartilage as a body tissue. Highly complex and delicate enzyme systems contained in the cartilage cell are involved in cartilage matrix formation and in the processes of calcification and cartilage repair. Heat, various drugs, freezing, and changes in the chemical environment damage or destroy these enzyme systems and interfere with the growth and function of cartilage. Hyaline cartilage to be transplanted must be handled with great care to preserve the cellular enzyme systems—otherwise the graft will be resorbed and clinical failure will result.**

WITHIN THE PAST DECADE, the literature pertaining to the chemical and physiologic features and the growth of cartilage has become voluminous and highly revealing. The increased activity in research has been due in large measure to the availability of research tools and methods not previously known, among them electron microscopy, radioisotope techniques, improved histochemical techniques and improved macrochemical and microchemical analytic and synthetic methods.

It is the purpose of this communication briefly to summarize some of the significant contributions resulting from these improved research methods during the past decade and to indicate the possible clinical importance in the fields of arthritis, cartilaginous tumors, congenital and growth deformities and, in particular, in the consideration of transplantation of hyalin cartilage.

### Carbohydrate Metabolism

The presence of large quantities of glycogen in the cytoplasm of cartilage cells was first described by Rouget[30] in 1859. His observation has been a factor in many theories including the following:

1. Glycogen derivatives through phosphorylation and transphosphorylation provide the substrates for phosphate esters utilized in calcification (Gutman and Yü).[21]
2. Glycogen, through its carbohydrate breakdown products, is a precursor of chondroitin sulfate in cartilage matrix (Zambotti).[35]
3. Glycolysis and the aerobic metabolism of carbohydrate may provide energy necessary for synthetic functions involved in the formation of collagen and of new bone at the epiphyseal line (Harper).[22]

Recent observations indicate that glycogen in cartilage is involved in all these functions and perhaps in others still to be discovered. The author's own observations recently presented[4] indicate that when rapidly growing cartilage is deprived of the normally available amount of glucose, the quantity of matrix formed about each cartilage cell is greatly reduced.

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Almost every enzyme involved in the process of anaerobic and aerobic glycogen metabolism has been detected in cartilage by various investigators (Table 1).

There is indirect evidence that other enzymes in the glycolytic cycles are also present in cartilage. Blocking experiments have indicated that triosephosphate isomerase and phosphoglyceromutase (triose mutase) are present in addition to those enzymes already identified.

The establishment of the presence of glycolysis in cartilage provides foundation for the assumption that glycogen is involved in the production of phosphate esters needed for calcification. It has been further shown by Gutman and Yü that calcification in cartilage cannot proceed when the glycolytic enzymes are inhibited.

An interesting sidelight of considerable importance is the demonstration by Tulpule and Patwardhan[33] that vitamin D is necessary in epiphyseal cartilage to facilitate the Krebs cycle oxidation of pyruvates. This indicates that vitamin D deficiency may actually operate by retardation of one or more steps in aerobic glycolysis, thus preventing the formation of substances essential for calcification.

Production of energy is a direct result of glycolysis, and of oxidation of carbohydrate in the Krebs cycle. These reactions take place in cartilage. It is not yet known how this energy is utilized.

### Synthesis of Chondroitin Sulfate and Sulfate Exchange in Cartilage

Chondroitin sulfate (Chart 1) is the principal carbohydrate component of cartilage matrix. It

TABLE 1.—*Glycolytic Enzymes Present in Epiphyseal Cartilage*

| Enzyme | Reference |
|---|---|
| Glycogen phosphorylase | Gutman and Gutman (1941)[20] |
| | Cobb (1953)[12] |
| Hexokinase | Gutman and Yü (1950)[21] |
| Phosphohexose-isomerase | Albaum, Hirschfield and Sobel (1952)[1] |
| Phosphohexose kinase | Albaum, Hirschfield and Sobel (1952)[1] |
| Aldolase | Albaum, Hirschfield and Sobel (1952)[1] |
| Triosephosphate dehydrogenase | Albaum, Hirschfield and Sobel (1952)[1] |
| Enolase | Albaum, Hirschfield and Sobel (1952)[1] |
| Lactic acid dehydrogenase | Albaum, Hirschfield and Sobel (1952)[1] |
| Citrogenase | Dixon and Perkins (1952)[15] |
| Aconitase | Dixon and Perkins (1952)[15] |
| Isocitric dehydrogenase | Dixon and Perkins (1952)[15] |
| | Follis and Melanotte (1956)[18] |
| Cocarboxylase | Zambotti and Lorenzi (1953)[34] |
| Coenzyme-A—DPN—dehydrogenase | Albaum et al. (1952) |
| Succinic acid dehydrogenase | Follis (1949)[17] |
| | Castellani and Zambotti (1954)[9] |
| | Follis and Melanotte (1956) |
| Malic-dehydrogenase | Follis and Melanotte (1956) |

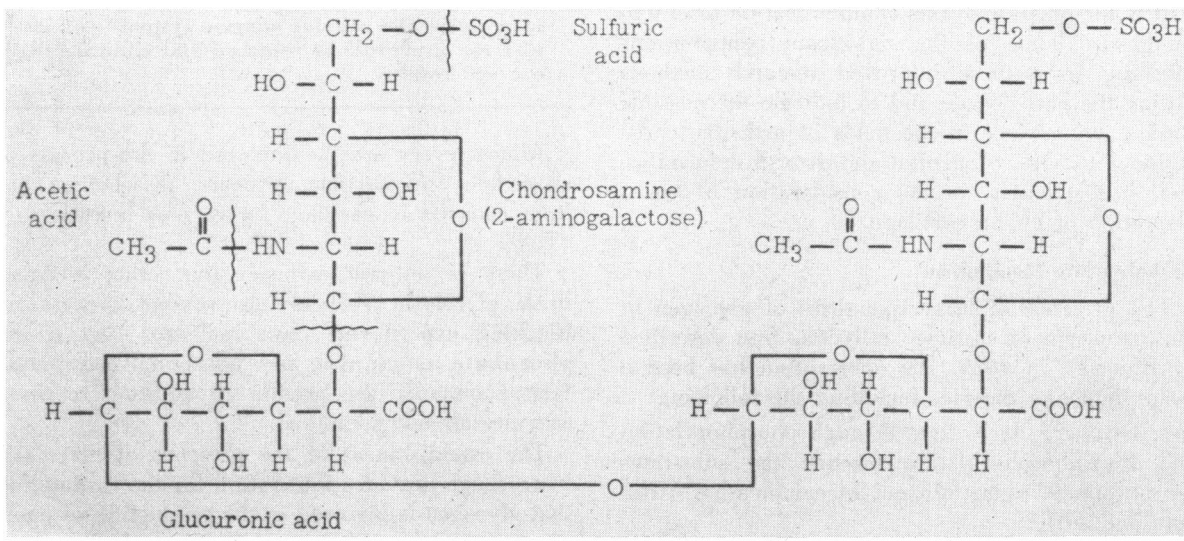


Chart 1.—Chondroitin sulfuric acid.

is composed of alternating units of sulfated acetylgalactosamine and glucuronic acid.[14] It occurs polymerized to a molecular weight varying between 100,000 and 2,600,000.

Approximately 10 per cent of chondroitin sulfate occurs as a free substance; the remainder is linked to protein. This protein is partly collagen and partly other nonperiodic and nonfibrous proteins. The nature of the bonds between chondroitin sulfate and protein are of importance because the strength and resiliency of cartilage appears to be directly related to the integrity of this bond. Cartilage becomes soft and loses its normal structure when treated with the enzymes trypsin and papain which destroy protein, or with hyaluronidase which hydrolyzes chondroitin sulfate.

Meyer,[25] who intensively studied cartilage proteins and their linkages, concluded that linkages of several types are probably present between matrix proteins and polysaccharides, but that salt linkages predominate. Such bonds are subject to cleavage by enzymes and by changes in hydrogen ion concentration.

Chondroitin sulfate is present especially in the immediate vicinity of the cartilage cell. This suggests that the cartilage cell is responsible for the production of chondroitin sulfate either by direct synthesis or by alteration of the surrounding tissue fluids in such a way as to bring about the deposition of chondroitin sulfate.

Evidence has rapidly accumulated to show that all the enzyme systems necessary for the synthesis of chondroitin sulfate are present in the cartilage cell and that these enzymes are more active in rapidly growing epiphyseal cartilage than in resting cartilage.

Castellani and Zambotti[10] in 1956 reported the presence of a thermolabile enzyme system in epiphyseal cartilage which catalyzes the synthesis of hexosamine from glutamine and glucose 6-phosphate. This process is ten times more active in epiphyseal than in tracheal or costal cartilage.

The synthesis of the glucuronic acid portion of chondroitin sulfate in cartilage was reported by Castellani in 1957.[11] This process involves the dehydrogenation of uridine-diphosphoglucose (UDP glucose) to uridine-diphosphoglucuronic acid (UDP glucuronic acid) in the presence of oxidized diphosphopyridine nucleotide (DPN). The UDP glucuronic acid is later split to UDP and glucuronic acid or conjugated with sulfated UDP-galactosamine to form the chondroitin sulfate molecule.

The various steps in the enzymatic synthesis of chondroitin sulfate from glycogen have been postulated by Zambotti and are set forth in Chart 2. This is slightly modified from a similar scheme published by Roden[29] in 1956.

Reactions 1, 2, 3 and 4 (Chart 2) are glycolytic cycle steps. Reaction 5 has been established by Castellani and Zambotti and 5a by Leloir and Cardini.[23] Reactions 7 and 8 were determined by Boström and Månnson[6] and by Brown[8] in 1953. Reactions 9 and 10, and 12 to 15 are concerned with uridine nucleotide and coenzyme transfers, several of which have been observed in cartilage and others observed by Glaser and Brown[19] in the synthesis of hyaluronic acid.

The exact mechanisms of sulfate fixation (reactions 11 and 12) are not entirely clear. The fact that fresh cartilage combines sulfate has been amply shown by Pelc,[28] Dziewiatkowski,[16] Boström and Månnson,[7] and Amprino.[3]

In this connection, the work of Boström and Månnson is of interest, as they studied with great detail the effects of many factors on the enzyme system responsible for the incorporation of labeled sulfate into chondroitin sulfuric acid.

Under carefully controlled experimental conditions they were able to determine the characteristics of the enzyme system involved in the sulfate incorporation into bovine nasal and costal cartilage. Briefly, the uptake of $S^{35}$-labeled sulfate by bovine cartilage is catalyzed by an enzyme system which is oxygen-dependent, temperature-dependent, and is destroyed by freezing and thawing, by heating, by damage to the cartilage cell and by enzyme inhibitors, especially the heavy metals (Table 2).

Amprino and Bélanger[5] showed that radioactive sulfur accumulates first in the cartilage cell, then in the matrix, suggesting again the direct synthesis of chondroitin sulfate by the cartilage cell.

The content of chondroitin sulfate in articular

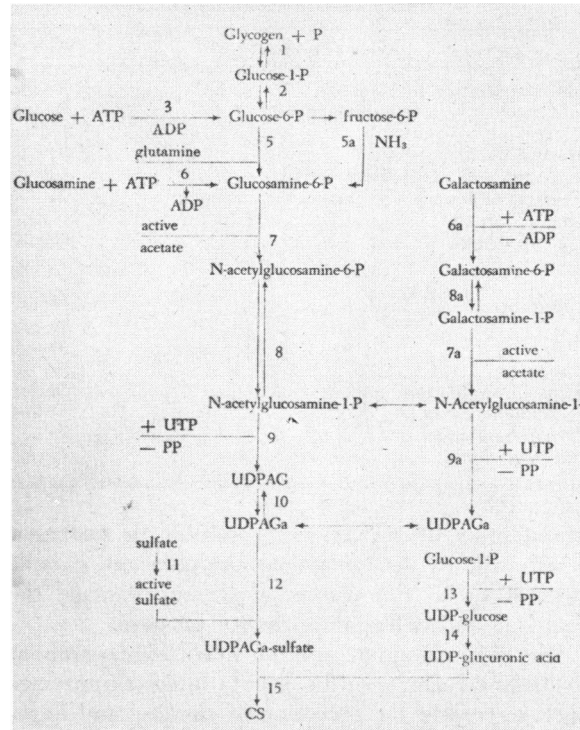


**Chart 2.—Possible pathways for the biosynthesis of chondroitin sulfate.**

P = phosphate
PP = pyrophosphate
ATP = adenosintriphosphate
ADP = adenosindiphosphate
UTP = uridintriphosphate
UDP = uridindiphosphate
UDPAG = uridindiphosphate-acetyl-glucosamine
UDPAGa = uridindiphosphate-acetyl-galactosamine
CS = chondroitin sulfate (or chondroitin sulfuric acid)

cartilage in humans has been found to diminish with advancing age and with degenerative joint disease.[24,24a] Under normal conditions the chondroitin sulfate content is higher in weight-bearing cartilage than in the upper extremities.

Amprino also showed that the radiosulfate once incorporated in skeletal cartilage in animals is not always permanently fixed but may be resorbed and incorporated in other areas of cartilage or even in the bone salt, emphasizing the fact that there is an active turnover of chondroitin sulfate in cartilage tissue. Even in fully differentiated cartilage, renewal of chondroitin sulfate in the matrix seems to occur.

### Clinical Applications

It is not within the scope of this communication to discuss in detail the clinical implications of the recent advances in knowledge of the chemical features and function of cartilage, yet certain observations may be of some value.

In trauma to joints and in arthritis the preservation of healthy articular cartilage is of prime importance. Since cartilage is not static, but is in a constant state of metabolic activity concerned with

TABLE 2.—*Characteristics of Cartilage Sulfatation Enzyme System (Boström and Månnson)*

| Agent or Process | Effect of $S^{35}O_4$ Uptake by Chondroitin Sulfate of Cartilage |
|---|---|
| Homogenization of cartilage | Reduced uptake by 67 to 93 per cent |
| Absence of $O_2$ in the atmosphere | Reduced uptake by 64 per cent |
| Increasing temperature | 100 per cent loss of uptake above 47° C. |
| Decreasing temperature | 50 per cent loss at 21° C.; 100 per cent loss at 0° C. |
| Freezing and thawing of cartilage | 100 per cent loss of uptake after freezing and thawing |
| Time after removal of cartilage from body | No loss up to 4 hours. Marked loss in 24-48 hours; total loss after 4 days. |
| Incubation time | Uptake most rapid in first 2 hours; tapers off after 10 hours |
| Age of animal | Uptake in 1-day-old calf ..... 100 per cent |
| | 2-year-old cow ..... 57 per cent |
| | 12-year-old cow ..... 26 per cent |
| Inhibitors | Iodoacetate—100 per cent inhibition in low concentration |
| | Mercurials—100 per cent inhibition in low concentration |
| | Arsenicals—100 per cent inhibition in low concentration |
| Penicillin | No loss at $10^{-3}$M/1 concentrations |
| Para-amino benzoic acid | No loss at $10^{-3}$M/1 concentrations |
| Sodium benzoate | No loss at $10^{-3}$M/1 concentrations |
| Cortisone alcohol | 30 per cent loss at $10^{-3}$ and $10^{-4}$M/1 concentrations |
| Salicylic acid | 30 per cent loss at $10^{-3}$ and $10^{-4}$M/1 concentrations |

maintaining its matrix, any condition or treatment which will be detrimental to the cartilage enzyme systems or to the matrix itself may damage the cartilage and defeat the primary purpose.

The indiscriminate use of hyaluronidase about articular structures or the use of proteolytic enzymes such as trypsin for reduction of swelling and fibrosis, while these have not been thoroughly studied, could conceivably produce harmful results. The injection of mercurials into joints results in the rapid destruction of articular cartilage. The long-term effects of the use of cortisone derivatives in diarthrodial joints is not known, and one may only guess at what effect many other drugs may have on the delicate enzyme systems concerned with the formation and maintenance of cartilage matrix.

With regard to cartilaginous tumors, very little can be said positively. Schajowicz and Cabrini[31,32] studied some of the histochemical alterations in chondromas and chondrosarcomas. The studies were very limited in scope, but they demonstrated the value of histochemical observations in understanding these neoplasms. It is hoped that further studies of the metabolism of abnormal cartilage tissue may broaden knowledge of these tumors and lead to new and effective methods of treatment.

Much has been written and much work has been done on metabolic considerations relating to congenital skeletal abnormalities. It is well established that agents that interfere with the glycolytic cycle (such as insulin, cortisone, sulfonamides and heavy metals) will produce congenital deformity in experimental animals. In humans, diabetes, vitamin deficiencies and starvation are statistically proven causes of stillbirths and of congenital deformities of the skeleton. It would appear that alterations in the embryonic circulation which are disadvantageous to the function of the cartilaginous enzyme systems during the period of chondrification and rapid cartilage growth of the embryo (in the period between the seventh and twelfth weeks of gestation) will interfere with formation of the skeletal cartilaginous anlagen and cause permanent defects and deformities.

Of possibly more direct concern to orthopedic surgeons are the implications of these findings on the use of cartilage as a transplantable tissue.

As recently as November, 1958, Allbrook and Kirkaldy-Willis[2] reported the use of preserved whale cartilage and of fixed decalcified autogenous and homogenous cartilage implants in the elbow joints of monkeys after radial head resection. It is not surprising that all these cartilage grafts were resorbed. The cartilage in this experiment was obviously unsuited for survival and its resorption was certain. This does not mean that all transplanted hyalin cartilage must undergo rapid resorption and replacement.

In July of 1958, Craigmyle[13] reported on long term cartilage grafts in rabbits. Two years after transplantation of fresh rib cartilage into subcutaneous tissue and muscle, heterogenous grafts were resorbed but autografts and homografts were found to have survived, the cartilage cells were still viable and the matrix still showed metachromasia and active $S^{35}$ uptake—equal to that of nontransplanted fresh cartilage controls.

In cartilage transplantation one of two results is desired: Either that the transplanted cartilage will live and function, or that the host tissues will, by metaplasia, form new cartilaginous surfaces as a result of the "inductive" forces of the transplant. There is ample evidence that viable cartilage will live and grow in other than its original position. This is seen in osteochondritis dissecans, in osteochondromata and in the case of the loose bodies in arthritis and following articular cartilage trauma. The case for the induction of cartilaginous meta-

plasia is not as strong, although this is observed in fracture callus, in synovial osteochondromatosis and in rare extra-skeletal cartilaginous tumors such as that recently reported by Murphy and Wilson.[27]

The factors responsible for cartilaginous metaplasia are poorly understood. On the other hand, the factors necessary for survival and function of cartilage grafts may now be stated with some certainty. From the information at hand, we may set down a theoretical list of rules for successful transposition of hyalin cartilage:

a. Heterogenous cartilage will not survive.

b. Autogenous cartilage is probably preferable to homogenous grafts.

c. Cartilage to be transplanted must not be subjected to freezing, nor to temperatures above 45° C.

d. Preservation of cartilage in strong antiseptic solutions is not permissible.

e. The graft should be used within a few hours of the time of its removal from the donor site.

f. The host tissues should be free of excessive trauma, hemorrhage and infection to permit adjustment of the graft to the host site with a minimum of alteration of chemical and cellular environment.

If the enzymes necessary for the formation of cartilage matrix are destroyed in the process of transplantation, or if the host environment is unfavorable for the function of these cellular enzyme systems, then the transplant will not survive in a healthy condition.

Clinical records are available of cases of cartilage grafts and transplants carried out in accordance with the theoretically derived rules listed above, and they bear out the validity of these dicta. Since 1938, J. R. Moore[26] of Philadelphia has employed the "cartilaginous cup arthroplasty" for ununited fractures of the neck of the femur, using fresh autogenous grafts of hyalin cartilage. Histological examination of a cartilage graft nine years after operation revealed the hyalin cartilage to be viable and to all appearances normal and basophilic. Other similar examples of completely successful transplantation of fresh autogenous hyalin cartilage are numerous. Moore still uses the cartilage cup arthroplasty after 20 years, and results in many cases are strikingly good. This appears to be an instance of the laboratory men's finally catching up with an outstanding clinician and surgeon to find belatedly that he has been doing the right thing all along.

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